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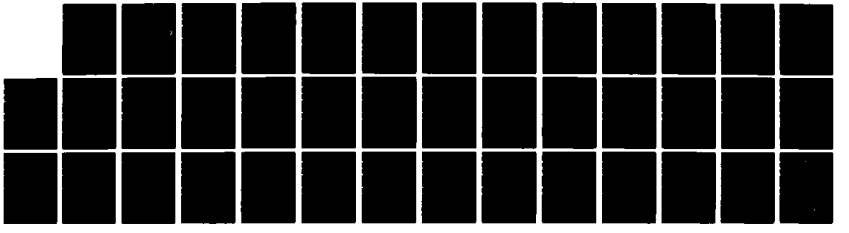

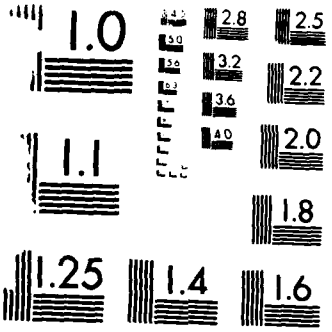

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FLUID-ELECTROLYTE-MINERAL INTERRELATIONS AS AFFECTING WORK PERFORMANCE

FINAL REPORT

MORTEZA JANGHORBANI, Ph.D.
ASSOCIATE PROFESSOR

NOVEMBER, 1985

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701-5012

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This is the Final report dealing with development of stable isotope approaches for the study of water-electrolyte dynamics in relation to exercise in heat.

This progress report describes the development of analytical chemistry of labeled water ($H_2{}^{18}O$) and rubidium (Rb) as tracers for the measurement of body water and its dynamics and intracellular mass and its correlates.

The methods used in these studies are based on Isotope Ratio Mass Spectrometry (IR/MS) and Inductively Coupled Plasma Mass Spectrometry (ICP/MS).

It is shown clearly that both these methods can be used effectively for simultaneous measurement of body water and body rubidium and their correlates in human adults.

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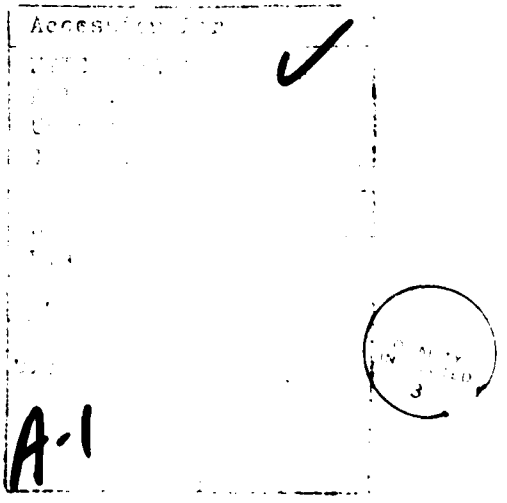

Foreword

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

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SUMMARY

This is the final report dealing with development of stable isotope approaches for the study of water-electrolyte dynamics in relation to exercise in heat.

This final report describes the development of analytical chemistry of labeled water ($H_2{}^{18}O$) and rubidium (Rb) as tracers for the measurement of body water and its dynamics and intracellular mass and its correlates.

The methods used in these studies are based on Isotope Ratio Mass Spectrometry (IR/MS) and Inductively Coupled Plasma Mass Spectrometry (ICP/MS).

It is shown that the analytical chemistry of the isotopes of oxygen and rubidium is developed sufficiently to permit exploration of their use as *in vivo* tracers in studies related to body water and body rubidium (potassium).

I. Introduction

The purpose of this project during the period of concern to this progress report was to initiate exploration of a number of important methodological developments related to stable isotopes for eventual use in studies of water-electrolyte balance. Initial studies have focussed on development of analytical chemistry of stable isotopes ^{18}O for measurement of total body water (TBW), stable isotopes of Rb to permit evaluation of the rubidium method as a tracer of Total Exchangeable Potassium (TEK), and stable isotopes of bromine to evaluate bromine space as a potential space to trace the behavior of Extracellular Fluid Volume (ECFV).

Following completion of these studies, we will test the concepts in appropriate animal model systems.

II. Analytical Chemistry Developments

A. Studies with $H_2{}^{18}O$. Application of the $H_2{}^{18}O$ principle to accurate measurement of TBW requires extremely precise measurement of the ratio $^{18}O/^{16}O$. This becomes clear when the following exercise is considered. If a 70-kg reference man contains 42 liters of body water, his content of ^{16}O and ^{18}O will be 2325.68 and 4.7793 g-atoms, respectively; the molar ratio of $^{18}O/^{16}O$ being 0.0020500. In a typical experiment, such a person would drink 40 grams of $H_2{}^{18}O$ with $^{18}O/^{16}O$ molar ratio of 0.181927. Assuming complete retention of the label and its uniform distribution in TBW, the molar ratio of $^{18}O/^{16}O$ after establishment of equilibrium will be 0.0021969. This is a 7.2% increase in the ratio compared with the natural ratio.

The equation employed to calculate TBW is:

$$TBW = \frac{k}{R_{18/16} - \overset{\circ}{R}_{18/16}} \qquad (1)$$

where k = constant

$\overset{\circ}{R}_{18/16}$ and $R_{18/16}$ = molar ratios of $^{18}O/^{16}O$ before and after dosing

The error inherent in the method due to the uncertainties of the two measurements ($\overset{\circ}{R}_{18/16}$ and $R_{18/16}$) can be determined from the following expression:

$$\frac{\sigma_{TBW}}{TBW} = \frac{\sqrt{\sigma_R^2 + \sigma_{R^\circ}^2}}{(R - R^\circ)} \qquad (2)$$

In Table 1, we have calculated the value of σ_{TBW}/TBW resulting from ingestion of either 40 g $H_2{}^{18}O$ or 10 g $H_2{}^{18}O$ and under two sets of measurement precision: a) $\sigma_R/R = \sigma_{R^\circ}/R^\circ = 0.1\%$ and b) $\sigma_R/R = \sigma_{R^\circ}/R^\circ = 0.01\%$. These calculations should clearly show the significance of precise measurements for the two ratios. If 40 g $H_2{}^{18}O$ is used and the measurements are carried out at 0.1% level of precision, we can see that the calculated value of TBW cannot be more precise than 2%; while for measurement precision of 0.1%, this value can be reduced by an order of magnitude (0.2%). This obviously has important implications in relation to the smallest change in dehydration status that can be accurately determined. Under the more stringent measurement conditions, we should be able to determine changes in TBW of better than 1%.

Table 1 - Calculated value of σ_{TBW}/TBW

g $H_2{}^{18}O$ administered*	Measurement Precision	
	0.1%	0.01%
40	0.020	0.0020
10	0.072	0.0072

*$^{18}O/^{16}O$ (molar ratio) = 0.181927

These issues are also important from the point of view of cost of isotope. The prevailing cost of $H_2{}^{18}O$ is about $3/gram. Therefore, in a large scale study involving, say, one hundred doses, administration of 40 g/dose would cost $12,000 while dosing at 10-g level would cost only $3,000.

We have addressed the issue of measurement precision by replicate analysis of the ratio $^{18}O^{16}O$ from a single source of demineralized water (Table 2). The analyses were carried out by a commercial laboratory and the reported values (δ-values) were converted to molar ratio for $^{18}O/^{16}O$ employing calibration plots of ^{18}O-spiked water. The results clearly establish that the delta-values can be reproduced to within 0.050 unit; the coefficient of variation for the ratios is 0.004%. Therefore, the method is highly reliable for measurements of small changes in TBW.

The Dynamic Linear Range of these measurements is important because the available isotope ratio instrumentation has been specifically designed for very precise measurement of isotope ratios close to SMOW (Standard Mean Ocean Water). In studies involving measurement of TBW and dynamics of water metabolism, the expected isotope enrichment is far in excess of these values. Therefore, accurate measurement of true ratios cannot be carried out unless appropriate calibration procedures are available. Employing matrices of water, urine, serum, red cells, and saliva, we have prepared ^{18}O-spiked standards covering the range of interest to human studies. The measured value of δ have been plotted against the calculated molar ratio of $^{18}O/^{16}O$ for these spiked-matrices in Fig. 1. The linear regression equations are given in Table 3. The results show a highly linear correlation between the two parameters. However, small differences appear to be present for different matrices. In this study, the water content of each sample was determined based on the weight of the sample and the known solid content of the matrix. Because water content was not measured directly, the observed differences in the parameters of the linear equations could be due to error of estimation of water content. This will be resolved. However, the absolute magnitude of the error involved in use of any of these four equations in the conversion of a measured value of δ to R is small. For instance, if δ for an unknown is 400, the maximum difference in the calculated ratio $R_{18/16}$ (using water and serum equations) will be about 0.2% of the value. Therefore, even if these differences are not taken into account, the resultant error is quite small.

Table 2 - Reproducibility of Isotope Ratio Measurements

Sample #	Measured δ,	$R_{18/16}$
1	-6.54	0.00205027
2	-6.65	0.00205006
3	-6.62	0.00205012
4	-6.53	0.00205029
5	-6.62	0.00205012
6	-6.61	0.00205014
7	-6.54	0.00205027
8	-6.65	0.00205006
mean $\pm$ 1SD	-6.595 $\pm$ 0.050	0.00205017 $\pm$ 0.000000084 (0.004%)

B. Studies with Rb. Rubidium possesses two stable isotopes, ^{85}Rb and ^{87}Rb. The natural abundances (weight %) are 71.70 and 28.30 respectively. These isotopes have not been employed previously in human studies.

We have developed a new method for accurate measurement of these two isotopes in matrices of interest to human studies. Below, we discuss those features of these developments whose understanding is essential to the next phase of the project, viz. application to human studies.

Table 3 - Linear Regression Equations for ^{18}O-Spiked Standards*

Matrix	A	B	r^2
Water	-1049.6	508742	.99993
Urine	-1030.2	501516	.99994
Serum	-1022.6	498300	.99999
Red Cells	-1026.9	500389	.99998
Saliva	-1020.4	498193	.99999

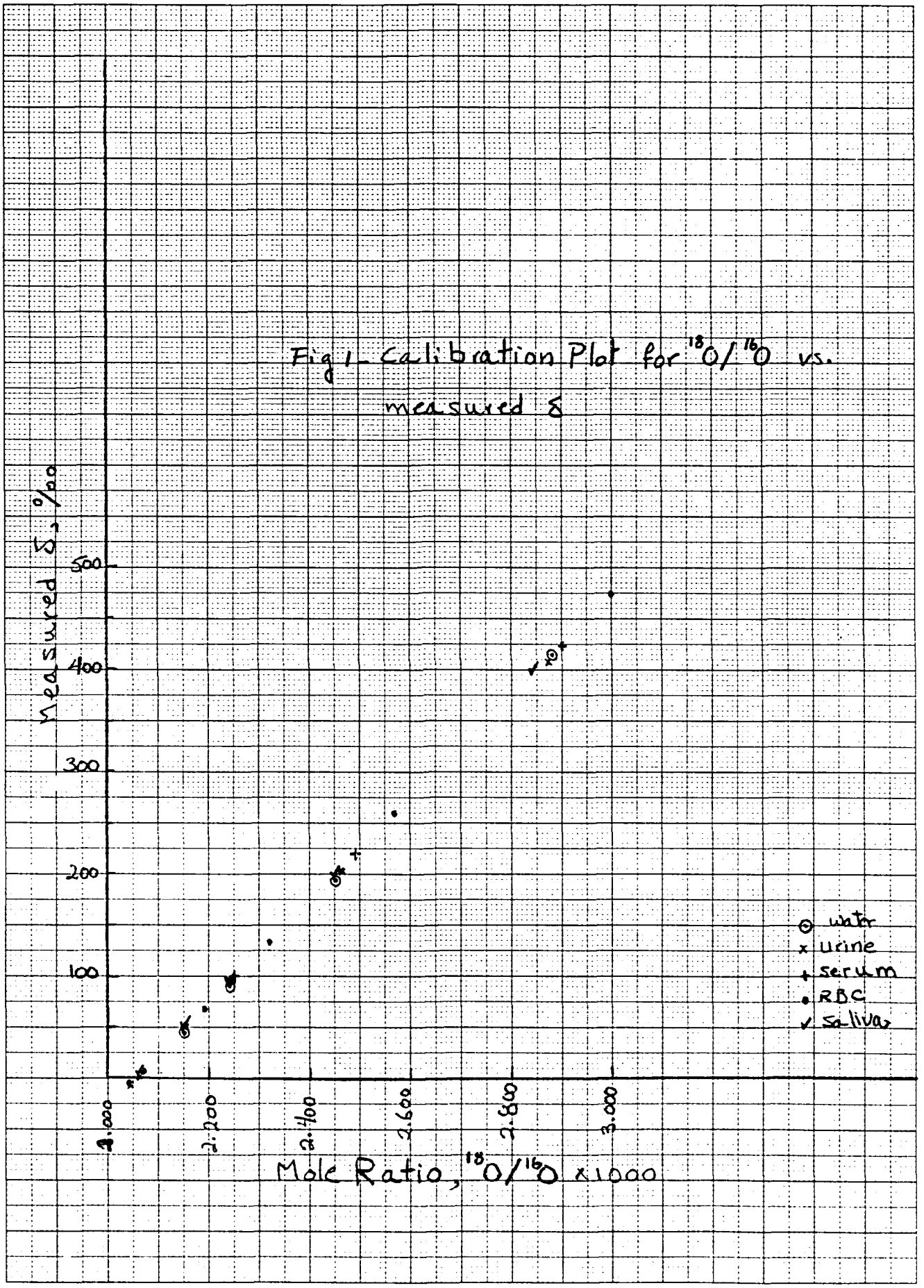
* $\delta_{,} = A + B.R_{18/16}$

1. Concepts. In order to use ^{87}Rb as a stable isotope tracer, an appropriate dose of ^{87}Rb is given orally or intravenously and its enrichment with respect to ^{85}Rb is determined in the matrix of interest (e.g. feces, blood, urine, or saliva). Total rubidium content is then measured, in the present study using AES. In the instance when this method is tested for possible use as a measure of TEK (Total Exchangeable Potassium) , elemental potassium is also measured, in this study again using AES (Atomic Emission Spectroscopy).

From the measured values of $^{87}Rb/^{85}Rb$ (called $MR_{87/85}$), true ratios, expressed on the mass scale, called $MIR_{87/85}$ (Mass Isotope Ratio), are calculated employing appropriate ^{87}Rb-spiked calibration standards. Combining the values of $MIR_{87/85}$ with total rubidium analyses, ^{87}Rb present in excess of its natural abundance is determined. From these data, we can additionally calculate TERb (Total Exchangeable Rubidium) and TEK_{Rb} (Total Exchangeable Potassium based on Rubidium). The various mathematical expressions required for these calculations are given below.

2. Calculations of ^{87}Rb excess. Since Rb consists of only two stable isotopes, the method of isotope dilution to determine absolute quantities of ^{85}Rb and ^{87}Rb in materials derived from in vivo studies with ^{87}Rb-label cannot be utilized unless two measurements (before and after in vitro respiking with ^{87}Rb) are carried out on the same sample. Although this can be done, a simpler alternative is to combine measurement of the ratio $^{87}Rb/^{85}Rb$ with analysis of total rubidium in the same sample. While this approach is relatively straightforward, there is the possibility of increased error depending on the relative precision and accuracy of the method of analysis for total Rb. Thus,

Fig 1 - Calibration Plot for $^{18}O/^{16}O$ vs. measured δ



the precision and accuracy of both $^{87}Rb/^{85}Rb$ and total Rb analysis combine to determine the overall precision and accuracy of the method.

Following administration of the $^{87}Rb_{label}$, the medium of interest is sampled and analyzed for two quantities: total rubidium (Rb_T) and the weight ratio of the two isotopes ($MIR_{87/85}$). The following two equations describe the quantitative relationship between Rb_T and $MIR_{87/85}$ and the two stable isotopes of rubidium ($^{85}Rb_n$, $^{87}Rb_n$: isotopes originating from natural element; $^{85}Rb_{label}$, $^{87}Rb_{label}$: the two isotopes originating from the label).

$$Rb_T = {}^{85}Rb_n + {}^{87}Rb_n + {}^{85}Rb_{label} + {}^{87}Rb_{label} \qquad (1)$$

$$= 1.395\ {}^{85}Rb_n + 1.020\ {}^{87}Rb_{label}$$

where the two constants reflect the isotopic composition of natural and enriched rubidium

$$MIR_{87/85} = \frac{{}^{87}Rb_n + {}^{87}Rb_{label}}{{}^{85}Rb_n + {}^{85}Rb_{label}} \qquad (2)$$

$$= \frac{0.395\,{}^{85}Rb_n + {}^{87}Rb_{label}}{{}^{85}Rb_n + 0.020\ {}^{87}Rb_{label}}$$

These two equations can be solved for $^{87}Rb_{label}$:

$$^{87}Rb_{label} = \frac{1.008\ (MIR_{87/85} - 0.395)\ Rb_T}{(1 + MIR_{87/85})} \qquad (3)$$

For any other enriched ^{87}Rb preparation, a similar expression would be used, containing an appropriate change in the constant.

An important consideration in tracer studies is the calculation of the statistical uncertainty associated with the calculated value of $^{87}Rb_{label}$. For any given set of experimental conditions, the smaller the administered dose, the closer the value of $MIR_{87/85}$ will be to the natural ratio, resulting in increased uncertainty in the value of $^{87}Rb_{label}$. The value of the uncertainty

associated with the calculated value of $^{87}Rb_{label}$ is given by the following expression:

$$\frac{\sigma_{Rb-87}}{^{87}Rb_{label}} = \sqrt{\frac{\sigma^2_{MIR}}{(1 + MIR)^2} + \frac{1.016\ Rb_T^2 \cdot \sigma^2_{MIR} + 1.016\ MIR^2 \cdot \sigma^2_{Rb_T} + .158\ \sigma^2_{Rb_T}}{(1.008\ MIR \cdot Rb_T - .398\ Rb_T)^2}} \qquad (4)$$

We have calculated some representative values for the parameter $\sigma_{Rb-87}/^{87}Rb_{label}$ and summarized these in Table 4. These calculations have been carried out for the red cell ($[Rb_T]$ = 4 μg/ml) for the three cases where the resultant mass isotope excess for ^{87}Rb after establishment of equilibrium is 10, 50, and 100% respectively. In an adult male whose total body rubidium might be 300 mg, this corresponds to ^{87}Rb dose level of 8.5, 42, and 85 mg respectively. These data clearly show the importance of precise measurements for both $MIR_{87/85}$ and Rb_T. For instance, if both these measurements are carried out at the precision level of 1% (coefficient of variation), administration of 8.5 mg ^{87}Rb will result in an expected uncertainty in the resultant value of isotope excess in the red cells of .185. In other words, we would expect our reported values of red cell isotope excess to be 10 ± 1.85%. If on the other hand, both measurements could be carried out at the precision level of .1% (which is not realistic), the expected value will be 10 ± .185%. These calculations point out the importance of the relationship between dose level and the required measurement precision. For instance, if there is no objection to administration of 85 mg ^{87}Rb in an adult male, there is little justification in requiring measurement precisions better than 1%. On the other hand, present price of ^{87}Rb (enrichment 98.0 at.%) is $4.20/mg Rb so that administration to an adult subject of 85 mg ^{87}Rb would cost $365. Thus, large-scale application could be costly. Under such conditions, a reduction in the required dose level by an order of magnitude could become highly desireable. The initial portion of the error function ($\sigma_{Rb-87}/^{87}Rb$ = f (dose)) drops rapidly in relation to increased level of dosing. For instance, under the conditions of 1% measurement precision for both $MIR_{87/85}$ and Rb_T, increasing the administered dose from 8.5 mg of ^{87}Rb to 25.5 mg would result in the value of the error function decreasing from 0.185 to 0.069 (Table 4). Thus, initially increases in dosing are effective in bringing about dramatic improvements in the reliability of the reported results, so that under the conditions where cost becomes an important consideration, careful evaluation of the overall performance of the method in relation to the needs of the study become necessary.

Table 4 - Calculated Values of $^{\sigma}Rb\text{-}87/^{87}Rb_{label}$ for Selected Values of Measurement Precision and Enrichment for Red Cell Matrix[1]

Assumed Mass Excess for ^{87}Rb (%)	$^{\sigma}Rb\text{-}87/^{87}Rb_{label}$ for Measurement Precision of (%)					
	ICP/MS	AES	ICP/MS	AES	ICP/MS	AES
	1	1	.1	1	.1	.1
10	.185		.149		.0185	
50	.047		.036		.0047	
100	.030		.022		.0030	

[1]Concentration of Rb_T: 4 μg/ml

3. Calculation of Total Exchangeable Rubidium (TERb). Total Exchangeable Rb (TERb) and its rate of exchange with the absorbed ^{87}Rb of the label can be determined from the appropriate isotope dilution equations (below) employing isotope enrichment data from blood or urine. There are no quantitative differences in data obtained from any tissue or fluid after the establishment of exchange equilibrium. The equation for calculation of TERb is:

$$TERb = \frac{1.395\ ^{87}Rb_{label,\ retained}}{MIR_{87/85} - MIR^{\circ}_{87/85}} \qquad (5)$$

where $MIR^{\circ}_{87/85}$ corresponds to the natural ratio of $^{87}Rb/^{85}Rb$. If it is assumed that excretion of the absorbed label via fecal and dermal routes is negligible, $^{87}Rb_{label,\ retained}$ equals the ingested $^{87}Rb_{label}$ minus the $^{87}Rb_{label}$ excreted in the urine. The magnitude of the latter is determined from analysis of urine following ingestion of the label and employing Eq. (3).

The precision and accuracy with which the value of TERb can be determined depend on the magnitudes of the corresponding errors associated with the terms

$^{87}Rb_{label,\ retained}$, $MIR_{87/85}$ and $MIR^{\circ}_{87/85}$. The last parameter, while constant in principle, is usually also measured so that its value also includes uncertainty. The mathematical expression necessary to determine the associated uncertainty of TERb is quantitatively similar to Eq. (4). The magnitude of the uncertainty, expressed as $\sigma_{TERb}/TERb$, can be determined accurately for any set of conditions. For instance, if we assume a dose of 85 $\pm$ .9 mg ^{87}Rb and equilibrium time of seven days (see sections below) for which $^{87}Rb_{label,\ retained}$ is 77 $\pm$ .9 (Eq. (4)) and the values for $MIR_{87/85}$ and $MIR_{87/85}$ of .7894 $\pm$ .0079 and .3947 $\pm$ .0039 we can readily show the calculated value of $\sigma_{TERb}/TERb$ to be 0.025. Thus, under these realistic conditions, it becomes evident that Total Exchangeable Rubidium can be determined with overall precision of 2-3%. This is in the same range as the overall performance that can be expected from radiotracer studies with ^{86}Rb.

4. Calculation of Total Exchangeable Potassium as Measured from Rubidium (TEK_{Rb}). This parameter is calculated as follows:

$$TEK_{Rb} = \frac{^{87}Rb_{label,\ retained}}{[^{87}Rb_{label}]/[K]} \qquad (6)$$

where, $[^{87}Rb_{label}]$ and [K] are concentrations respectively of the mass excess ^{87}Rb and K in the matrix used for analysis (red cells). This equation is applicable if it is assumed that the ratio of concentrations of $^{87}Rb_{label}$ to K in the medium of analysis is representative of the average value for the entire potassium space. This issue is a controversial matter at this time and its validity appears to depend strongly on the matrix of analysis and the conditions of the study.

5. Analytical Chemistry of Rubidium (Isotopes). Two methods appear to be potentially applicable to quantitative measurement of isotope ratio $^{87}Rb/^{85}Rb$: ICP/MS and TI/MS. We have pursued both these techniques and here provide comparative data on their performance.

For the measurements with TI/MS, high potassium content interferes with Rb measurements. A workable K/Rb ratio is about 10/1. In comparison, natural ratios of K/Rb in urine and red cells are about 1000/1. For ICP/MS, while it is possible to aspirate unprocessed body fluids directly into the argon plasma, the high salt content may cause instrument instability. Thus, a modest separation of matrix constituents is deemed necessary. These considerations have led us to development of a suitable separation scheme which yields samples for introduction into both ICP/MS and TI/MS (Fig. 2).

Measurements with TI/MS. Data on reproducibility of measurements of the ratio $^{87}Rb/^{85}Rb$ are given for TI/MS in Table 5 for a number of materials. As indicated in the table, the reported values for each sample type represent the mean $\pm$ 1SD for the number of replications (actual sample loadings using an automatic multiple sample turrets system) indicated. These data indicate that under the conditions employed, the coefficient of variation for these measurements was in the range 0.2 - 0.5%.

Measurements with ICP/MS. TI/MS is a well established method of isotopic analysis in geochemistry. In contrast, ICP/MS is a new technique and its ability to accurately measure $^{87}Rb/^{85}Rb$ in biological samples has not been previously evaluated. As this is the first report of these measurements in relation to the present application, we have provided the necessary data to permit realistic evaluation of its performance characteristics.

Mass spectra generated from the argon-plasma possess special spectral characteristics which could become limiting in stable isotope tracer studies. Of the three types of potential mass spectral interferences involved with ICP/MS; viz. argon-plasma mass spectra, reagent generated mass spectra, and isobaric mass spectra, the former two do not appear to present any significant problem for the measurements of ^{85}Rb and ^{87}Rb. The only known potential isobaric interference is that from ^{87}Sr (nat. ab. 7.0 at.%). However, we have established that the strontium contribution to ^{87}Rb is negligible. The ion intensity observed for ^{87}Rb in a typical run was about 30,000 ions/sec. In comparison, even for the major-abundant strontium isotope (^{88}Sr, nat. ab. 82.6 at.%) the ion intensity is negligibly small compared to that observed for ^{87}Rb. Similar results have been observed for red cells.

Precision of isotope ratio measurements for $^{87}Rb/^{85}Rb$ using the ICP/MS was tested by replicate analysis of natural and ^{87}Rb-spiked samples (Table 6). Each sample was processed according to the scheme given in Fig. 2. The intrasample precision (1SD of ten sequential ratio measurements on a single solution) of these measurements was about 1%. The data show clearly that intersample coefficient of variation was in the range 0.3 - 0.8 % (cf. data of Table 5 for TI/MS).

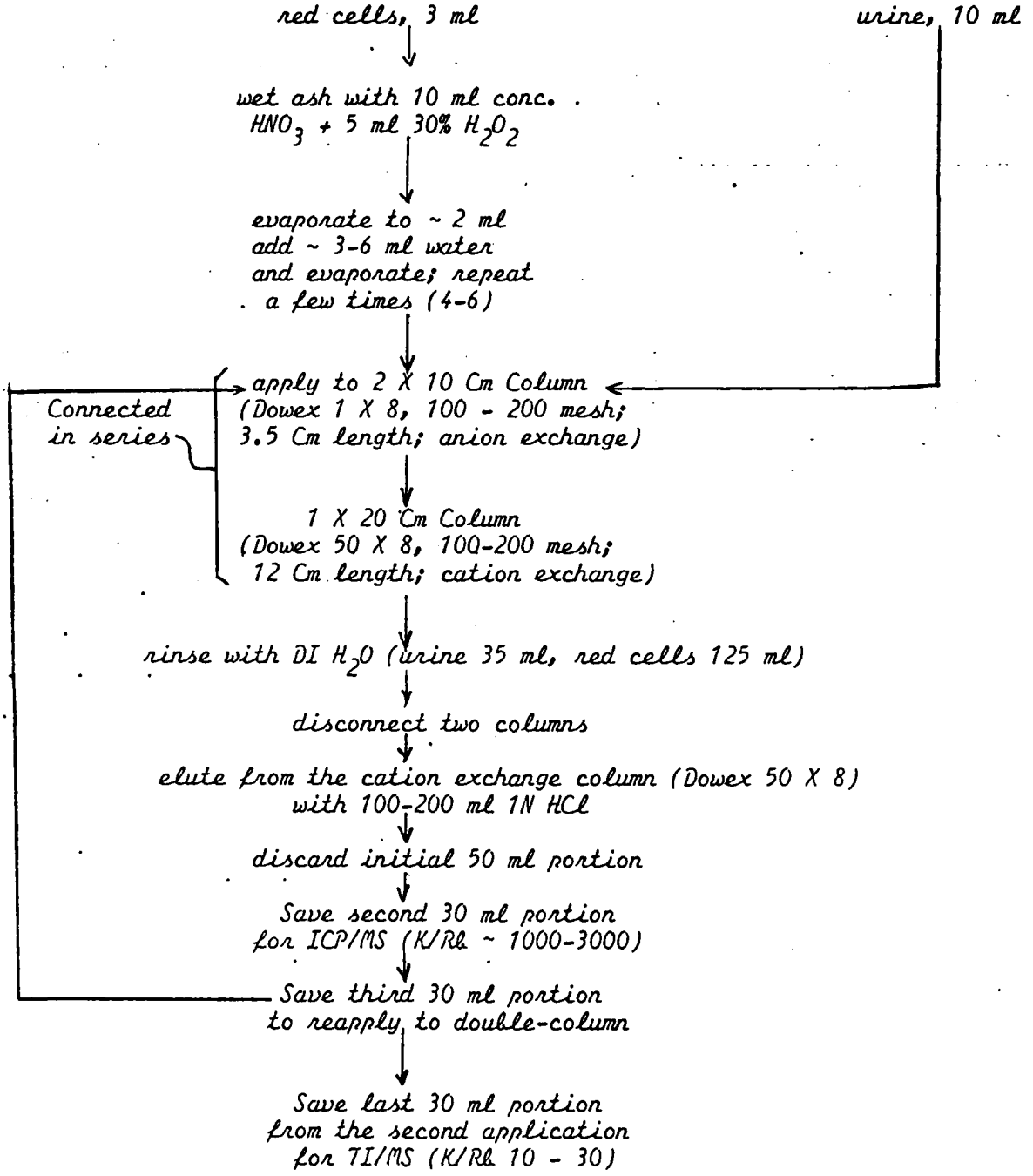


Fig. 2 - Separation Scheme for Rb Isotopes

Table 5 - Precision of Isotope Ratio Measurements for $^{87}Rb/^{85}Rb$ with TI/MS[1]

Matrix	# Sample Loadings (n)	$^{87}Rb/^{85}Rb$ (mean + 1SD)
Standard Solution	6	.3895
of RbCl		.0020
RbCl (Fisher Scientific)	9	.3903
		.0009
Rb_2SO4 (K & K Labs)	8	.3876
		.0013

[1]Measurements carried out by Dr. Harold Krueger (Krueger Enterprises, Cambridge, MA)

Table 6 - Intersample Precision of ICP/MS

Sample ID	n	$MR_{87/85}$	SD
Urine, Natural	6	.3888	.0013
Urine, enriched	6	.8168	.0040
Red Cell, Natural	6	.3855	.0028
Red Cell, enriched	6	.8524	.0043
HCl + Rb natural	6	.4019	.0036
HCl + Rb enriched	5	.7786	.0027

Employing graded ^{87}Rb-spiked samples of synthetic solutions, urine, or red cells, calibration plots expressing the relationship between the expected isotope ratio ($MIR_{87/85}$) and the measured ratio ($MR_{87/85}$) were constructed. These plots are given in Fig. 3. The linear regression equations are given in the legend of Fig. 3. It is clear that highly linear calibration plots can be constructed. However, it is not clear from the present data whether these plots differ in detail for the various matrices of interest and whether matrix matching is actually required. This is because the calculated values of $MIR_{87/85}$ are uncertain to the extent that our analysis of red cell or urine rubidium may be in error (see section below). The maximum error resulting from any potential matrix effect can be readily estimated by considering the calculated values of $MIR_{87/85}$ at the upper end of enrichment, e.g. $MR_{87/85}$ of 0.80, between plots (a) and (c). For such a case, using plot (a) at $MR_{87/85}$ of 0.80 we would estimate the value of $MIR_{87/85}$ at .7447 while for plot (c) the corresponding value would be .7920, a 6.2% difference. In practice, calibration plots are made from the same matrix as the samples so that this issue does not introduce a source of concern. The dynamic linear range of the $MR_{87/85}$ vs. $MIR_{87/85}$ plots is much greater than indicated in Fig. 3. The range of $MIR_{87/85}$ employed for the construction of plots in Fig. 3 was chosen because in practice we do not expect to exceed the upper level of represented enrichment; 100% mass excess of ^{87}Rb (dose = 85 mg) would correspond to $MIR_{87/85}$ of 0.789. However, we have tested the linearity over the range 0.3947 - 3.445 and find no evidence of nonlinearity. This is shown in Fig. 4 for the 1NHCl matrix (plot (a) of Fig. 3). There are virtually no differences between the two linear plots ((a) of Fig. 3 and Fig. 4) indicating the excellent linear dynamic range of these plots well beyond the range of any real value to human studies.

Comparative Performance of TI/MS and ICP/MS. Employing the scheme of Fig. 2, ^{87}Rb-spiked urine and red cells were analyzed for their isotope ratio with both ICP/MS and TI/MS. The results have been plotted in Fig. 5 and indicate excellent agreement between the two methods. Thus, it is clear that TI/MS, as applied in this study, may possess somewhat better precision (Table 5; observed range of coeff. of variation 0.2 - 0.5%) than that corresponding to ICP/MS (Table 6; observed range of coeff. of variation 0.3 - 0.8%). However, under the conditions of this study, the observed precision performance of TI/MS is not dramatically superior to that for ICP/MS. The outcome of the somewhat improved precision in regard to its effect on the uncertainties of the estimates of rubidium space (Eqs. (3) - (5)) is practically negligible. The measured values of $MR_{87/85}$ in urine and red cells processed by a common scheme (Fig. 2) are practically identical between the two methods (Fig. 5). Thus, in terms of instrument performance, either method is as suitable for these measurements.

As clearly evident from the scheme of Fig. 2, application of TI/MS requires considerably greater separation of Rb from K. In contrast, ICP/MS does not possess a stringent separation requirement. In fact, we have previously reported satisfactory application of the method to the measurement of stable isotopes of iron in whole blood without any chemical separations. In the

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Associate Professor

Fig. 3 - Calibration Plot for $MR_{87/85}$ vs. $MIR_{87/85}$

(a) $MR = 1.1397(MIR) - .04878$
$r^2 = .9999$

(b) $MR = 1.1147(MIR) - .0522$
$r^2 = .9993$

(c) $MR = 1.0447(MIR) - .0274$
$r^2 = .9995$

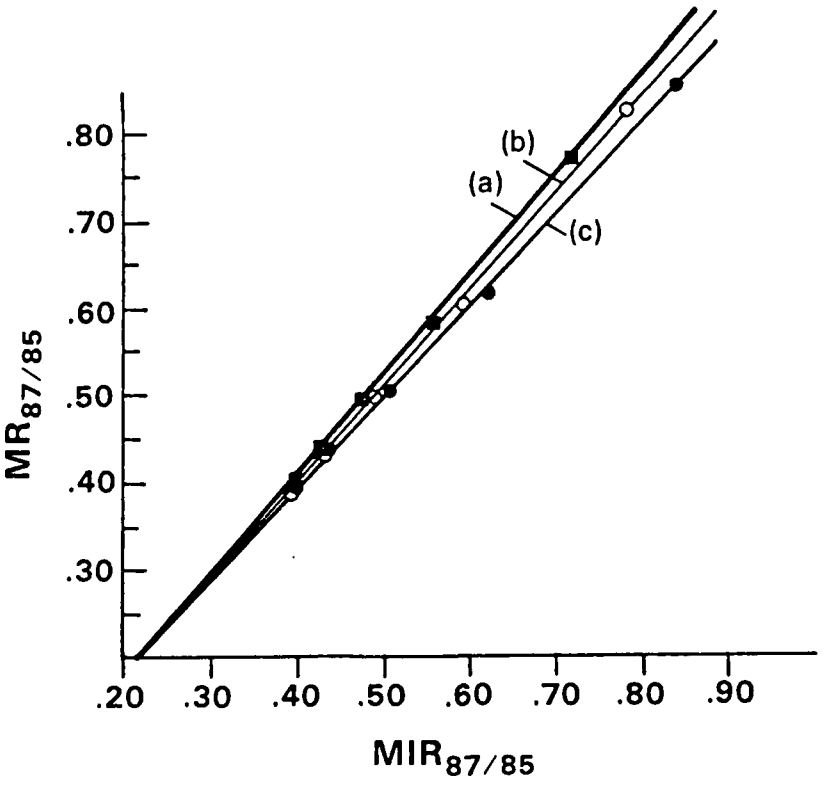

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Fig. 4 - Extended Dynamic Linear Plot for $^{MR}_{87/85}$ vs. $^{MIR}_{87/85}$

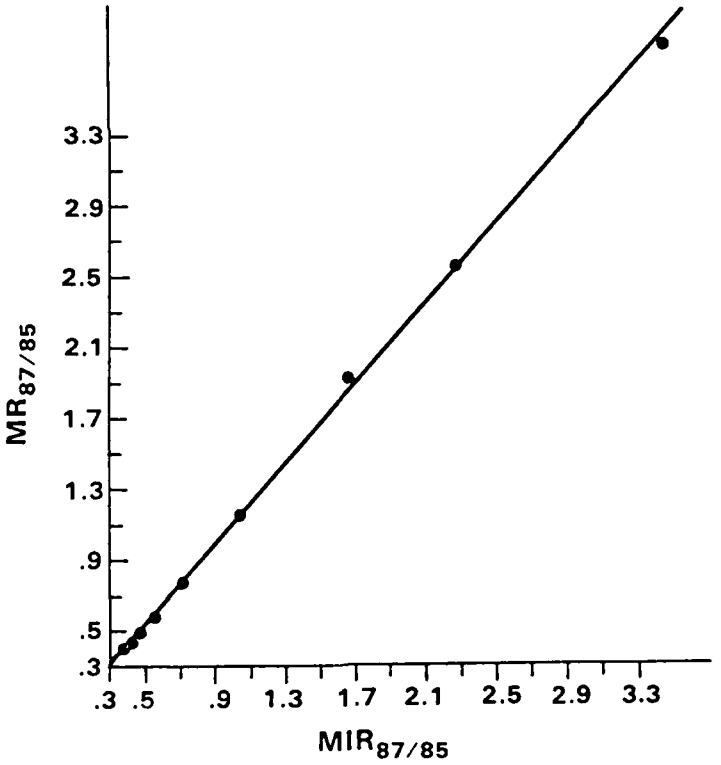

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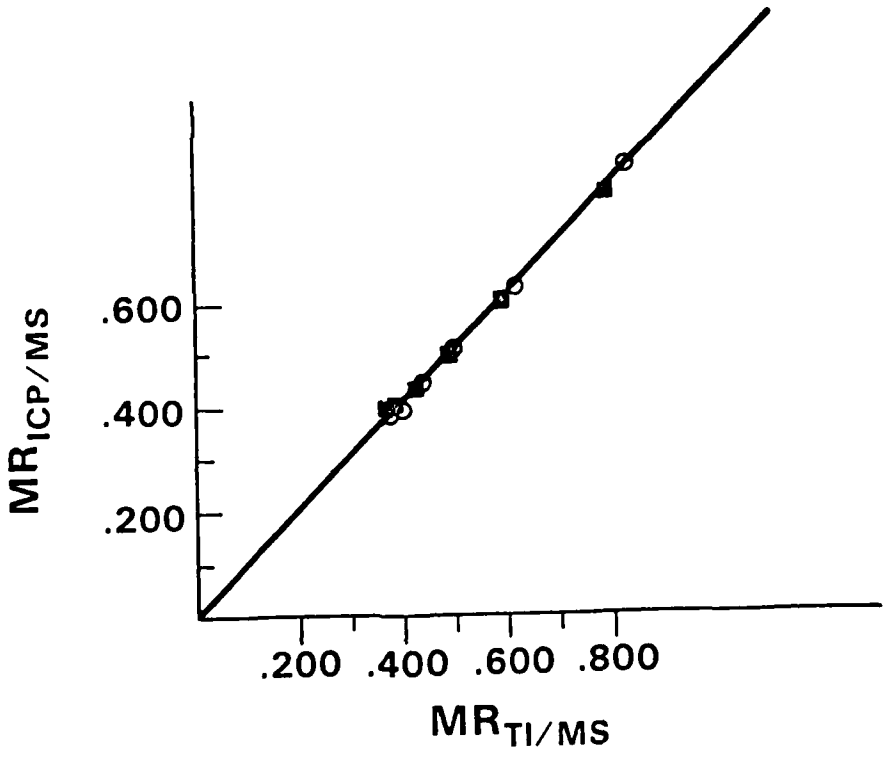
Fig. 5 - Comparison between Measured Isotope Ratio (MR) in Samples of Urine (x) and Red Cells (o) Spiked with ^{87}Rb for ICP/MS and TI/MS



present application, we have chosen a limited chemical separation as we are concerned about potential effects of high-salt solutions on the long-term performance of the instrument.

Accuracy of Rb Analyses. We have tested the issue of accuracy by analyzing samples of human urine for absolute Rb content by three methods: Isotope Dilution Analysis (IDA) employing ^{87}Rb as *in vitro* spike and ICP/MS; 2) Standard Addition using atomic emission spectrophotometry; and 3) Standard Curve employing atomic emission spectrophotometry. In the IDA method, the individual values of MR were converted to MIR using spiked urine standard samples (similar to plot (b) of Fig. 3). The results of these analyses are given in Table 7. All methods resulted in similar values for urine Rb concentration. Therefore, the standard curve method of atomic emission spectrophotometry, the fastest and simplest technique, was used for subsequent determinations of total Rb.

Studies with Bromine. We have begun development of chemistry of stable isotopes of Br. We have not yet made sufficient progress in this regard, but our preliminary data indicate that we should not expect any unsurmountable obstacles in these developments.

III. Future Directions

Studies with $H_2{}^{18}O$. Our analytical chemistry of ^{18}O is now ready for application to human studies.

Studies with Rb. We are now proceeding to initiate animal model studies to validate the concepts involved in the eventual application of Rb isotopes to human studies.

Studies with Br. We intend to complete analytical chemistry of Br isotopes, similar to the successful developments for Rb. Following this, we will then proceed to validate the necessary concepts in animal model systems.

Table 7 - Data Comparing Accuracy of Rb Analyses

Method	# Replications	ppm Rb ± 1SD
IDA	10	1.202 ± .038
Standard Curve	10	1.213 ± .015
Standard Addition	4	1.154 ± .068

IV. Instrument Development

Following successful development of our initial procedures (described above), we have been awarded the funds to acquire a high-precision isotope ratio instrument to complement our present capabilities. After in-depth search for

optimum instrument, we have come to the conclusion that the long-term objectives of our projects in the area of water-electrolyte metabolism can best be served if a special instrument were constructed specifically for these applications. We have now received approval from USAMRDC to proceed with this and have entered into formal agreement with the Nuclide Corporation (see attached appendix) for construction of such an instrument. Additionally, Boston University School of Medicine has agreed to provide the necessary funds for laboratory renovations which are required for this instrument. It should be pointed out at this time that this instrument will provide a broad capability not in existence today in any single instrument. This is extremely important for future needs of projects dealing with metabolism of water and electrolytes.

V. Literature Search

Literature of isotopic methods applied to body composition is extensive. Two books are available which have summarized the relevant literature (Moore, etal, 1963; Cheek, 1968). However, the majority of the literature has dealt with the application of radiotracers to dynamic measurements of body composition. These are clearly not applicable to the proposed studies based on the stable isotope tracer technology, except for background information.

In this report, we have included the results of our literature search dealing with the available information on the relevant aspects of the isotope tracer technology. We have also included the portion of literature serving as the basis for the fundamental concepts involved in the application of in vivo tracers to those studies of body composition relevant to the purposes of our studies. The citation has been organized according to the main theme of the articles (measurement of total body water, body potassium, body bromine, blood volume and simultaneous measurements).

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Articles

Measurement of Body Water

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Simultaneous Measurements

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VI. Appendix

This appendix consists of the following: Page No.*

* The page numbers denotes the page number of this report, numbering consecutively from the Summary to last page of the report.

SPECIFICATIONS

B.U.M.C. P.O. 15281-4706-X

Special Two-Analyzer IRMS System for Gases with Capability for Upgrade to Solid Samples.

General Description:

A two analyzer system, consisting of a 12-90-RMS system for $^{18}O/^{16}O$ isotope ratio analysis as CO_2 and a 3-60-HD for D/H isotope ratio analysis as H_2. The two analyzers will be mounted with a common gas inlet, common source vacuum pump, and common electronics. The design, however, will permit possible upgrade to two separate IRMS systems that can be run at the same time should the Buyer elect to purchase additional inlets, pumps, and electronics at some future time. The 12-90-RMS system shall also be designed to permit immediate upgrading to a solids inlet system with thermal ionization for the isotopic analysis of elements such as potassium, calcium and rubidium.

Performance verification:

Oxygen and hydrogen isotope ratio analyses shall be verified at plant and at site to meet or exceed the below-listed Performance Specifications:

Shipping and insurance:

To be provided by vendor.

Installation and training:

To be performed at buyer's site, and provided by vendor.

Vacuum system:

Both analyzers (with the exception of the turbo pump) shall be bakeable to 200°C. The metal parts shall be joined by TIG welds or vacuum brazing. The vacuum systems shall be free of leaks and capable attaining and maintaining a base pressure of 2×10^{-8} torr after bakeout and 48 h of pumping.

3-60-HD: Pump shall be connected to the source housing via an isolation valve capable of holding at least 1×10^{-7} torr against atmospheric pressure. Pump shall be capable of maintaining MS working pressures at or below 1×10^{-6} torr at the gas flows described below.

12-90-RMS: This unit will be differentially pumped. Source pump shall be connected via an isolation valve capable of holding at least 1×10^{-7} torr against atmospheric pressure. The source pump shall be capable of maintaining a working pressure at or below 7×10^{-7} torr at flows described below. The analyzer pump shall be capable of maintaining a working pressure at or below 1×10^{-7} torr under the same conditions.

Gas inlet (Interpolative):

The inlet shall consist of three variable-volume reservoirs. Minimum volume of the reservoirs shall be less than 3 ml. The reservoirs shall be connected to the switching valves via capillary leaks with a maximum internal diameter of 0.007 inches. The leaks shall be equipped with individual crimping devices to control flow. The switching valves shall be metal bellows valves and cross-talk (i.e. the total contribution from the two samples flowing to the waste pump to the sample flowing to the mass spectrometer) shall be less than 0.05%. The waste pump shall be independent of the pumps on the analyzer, and capable of a base pressure of 5×10^{-8} torr and a working pressure of 1×10^{-6} torr. This inlet shall be connected to both analyzers with an appropriate manifold and independent isolation valves. The manifold shall use minimal plumbing, such that the switching delay between gases need not exceed 20 sec - i.e. the intensity of the major ion falls to less than 0.1% of its working voltage within 20 sec of diverting all gases to the waste pump.

Ion Sources:

3-60-HD: Electron bombardment, with gas inlet from switching valves.

12-90 RMS: Electron bombardment with gas inlet from switching valves. In addition, a triple filament vaporizer/ionizer for solids shall be supplied. Modifications are to include a filament viewing port, a LN2 trap, and a sliding bar "SU" vacuum lock with vacuum system.

Collectors:

3-60 HD: Two modes of dual Faraday cup/multicollector collection to be provided (a) for D/H analysis of H_2, and (b) for $^{18}O/^{16}O$ or $^{13}C/^{12}C$ analysis of CO_2.

12-90 RMS: Type III RMS/SU triple Faraday cup detector with externally variable slits and rotation for analysis of m/z 44-45-46 or 28-29-30, and also an electron multiplier.

Electronics:

Electronics shall be solid state and fully detailed in accompanying schematics. Electronics and connectors shall be fully compatible with both analyzers so that either analyzer may be operated from the common electronic consoles, but not at the same time.

Ion source and electron multiplier controller: MEC-4 to meet or exceed specifications in Nuclide Pubs. 1852-0878.

Ion current amplifiers: meet or exceed the EAH-300 specifications in Nuclide pubs 1117-A-0873. Major ion feedback resistor of 1×10^{10} ohm, minor ion feedback resistors of either 2 or 5×10^{11} ohms as decided.

Electromagnet controller: meet or exceed specifications in Nuclide Pubs 1151-0771.

Ratiometer: IR-6-05, integrating digital ratiometer that shall be capable of measuring two ratios of three ion beams simultaneously and meet or exceed specifications in Nuclide Pubs 2101-0182.

Data system: IMB-PC based PC/GIRD with data system to control valve switching, intensity matching, isotope ratio data collection, and reduction of data to isotopic abundance of ^{18}O or D. Additional PC-SIM program for peak stepping isotope ratio analysis.

Performance Specifications:

3-60 HD:

General operating conditions: inlet reservoir 50-80 torr H_2, viscous flow approx. 1.5×10^{-9} moles/sec, source region pressure at or below 1×10^{-6} torr, delay between gas switching less than 20 sec. Integration time per pair of gases less than 5 min total. Ionization efficiency of approx. 1.3×10^{-5} ion/molecule shall provide a m/z 2 ion current of 2×10^{-9} A. Triprotium contribution at m/z 3 shall be less than 2×10^{-13} A or 50 ppm D equivalent. A series of 10 consecutive five-minute isotope ratio difference measurements of two gases (reference and sample gas) shall have a standard deviation of 1°/oo of the isotopic difference (i.e. the difference shall be measured to ±1 per mil - for example Δ = 10 ± 1 per mil).

12-90 RMS:

General operating conditions: inlet reservoir 20-40 torr CO_2, viscous flow at or below 1×10^{-9} moles/sec, source pressure at or below 7×10^{-7} torr, delay between gas switching less than 20 sec, and integration time per pair of gases less than 5 min total. Ionization efficiency shall exceed 2×10^{-5} ions/molecule and thus provide a m/z 44 ion current of 2×10^{-9} A. Abundance sensitivity shall be less than 1×10^{-13} A at m/z 45 and 2×10^{-14} A at m/z 46. This corresponds to abundance sensitivity corrections of 1.005 and 1.003 for ^{13}C and ^{18}O, respectively. A series of 10 consecutive five minute isotope ratio difference measurements of two gases shall have standard deviations of less than 0.1 °/oo for both ^{13}C and ^{18}O.

Notes:

1. Definition of °/oo, "per mil" $=((R_U/R_S)-1)1000$
where R is the ratio of the minor to major ion of the unknown and standard gases.

2. Alternatively, the interpolative method (with bracketing standards) may be used, at Seller's election, and if so the time per measurement stated above shall be increased to 7.5 min.

Definition of standard deviation = $\sqrt{\frac{\Sigma(X_i - \bar{X})^2}{n-1}}$
where x_i are the individual 5 minute isotopic ratio difference measurements and n is the number of observations; expressed as part per thousand of the mean.

Demonstration of (consignment) thermionic source of 12-90-RMS/SU

Nuclide will demonstrate that it is possible to achieve a standard deviation of +0.5 per mil for the ratio $^{41}K/^{39}K$ (at natural abundance) when measured using an elegant technique like that described by Gramlich et al (Clin. Chem. 28 (1982), 1309-13).

B.U.M.C. P.O. 15281-4706-X

Addendum to Special Terms of Purchase

(10 Sept. 85)

1. Delivery and Acceptance: The completed instrument shall have been installed on the buyer's premises and appropriate acceptance tests completed no later than February 28, 1986. The acceptance tests to have been completed by the said date, in addition to the Seller's standard instrument tests, include repetitive measurements for $^{18}O/^{16}O$ and $^{2}H/^{1}H$, as specified on pages 3 and 4 of the Specifications attached to this document.

2. Late completion: Should the system not meet certain specifications by 28 Feb., 1986, a penalty will apply. It's amount will be computed as 0.1% of the value of the portion not yet accepted, per (B.U.M.C.) normal working day. (Thus, the maximum penalty is $162/day.) Saidpenalty shall accrue and shall either be deducted from the final payment due, or, at Buyer's option, may be used as a credit toward payment for additional Nuclide products or services.

3. The quoted price in quotation Q-8-40-85-A, includes on-site installation, and one-week training for one person either on-site or at the Vendor's facilities.

4. The instrument shall be complete with the necessary data collection processor, including all the necessary system software.

5. Both parties agree that should the Vendor (Nuclide Corporation) not be able to complete delivery of the completed systems because of bankruptcy or other cause prior to the delivery date of February 28, 1986, all components paid for by the Buyer per the "progress payment" terms of this general agreement shall become the exclusive property of the Buyer and shall be delivered to the Buyer, upon request.

6. The thermionic source performance demonstration given on Pg. 4 of the Specifications need not be performed by 28 February 1986. It will be scheduled at a mutually convenient time, before 30 April 1986.

Nuclide Corporation

L. F. Herzog

Boston Univ. School of Medicine

Morteza Janghorbani

B.U.M.C. P.O. 15281-4706-X

SPECIAL TERMS OF PURCHASE

(10 Sept. 85)

1. Nuclide agrees to supply for the agreed price of $162,640 the MS system described in Nuclide's Quotation Q-8-40-85A (attached), in Sections IA and IB (pg. 1), and further in Nuclide publication 1927 (also attached).

2. Nuclide further agrees to supply the said system for the price stated, and also to furnish on a one-year "consignment" basis the items listed in sections II, A, B, and C (pg. 2) of the referenced quotation, plus a system for extracting hydrogen from water.

3. B.U.M.C. agrees to make a payment of $16,000 within 5 working days of the date of provisional order (15 August 1985).

4. B.U.M.C. further agrees to make additional payments on a "monthly progress payment basis", commencing on 15 September 1985, for 85% of the total value of work completed (as certified by Nuclide using its standard, Gov't approved progress reporting form), with 15% retained until completion; however, the total amount paid before delivery shall not exceed 75% of the total price. The balance will be paid as items are delivered and installed and demonstrate acceptable performance.

5. BUMC has the right to depute a representative to verify progress at any mutually convenient time; however, BUMC need not exercise this right, and may instead rely on Nuclide's certification, per 4 above.

6. This Agreement supercedes a Provisional Purchase Agreement the parties executed on 15 August 1985.

7. Changes in this Agreement may be made and appropriate Addenda added, by mutual agreement. Accordingly, an Addendum dated 10 Sept. 85 is hereby incorporated in the Agreement.

Nuclide Corporation

Boston Univ. School of Medicine

E: (814) 238-0541

CABLE: NUCLID

NUCLIDE CORPORATION

642 EAST COLLEGE AVENUE
STATE COLLEGE, PENNSYLVANIA, U.S.A. 16801

DATE August 14, 1935

TO
Dr. Morteza Janghorbani
Boston University
School of Medicine
85 East Newton Street
Room M-1008
Boston, MA 02118

OUR QUOTATION NO. Q-8-40-85-A

YOUR REFERENCE NO.

ITEM	QUANTITY	DESCRIPTION	UNIT PRICE	EXTENSION
		I. Special Tandem Analyzer IRMS System for Gases with capability to upgrade for solid samples also		
		A. 12-90-RMS System for ^{18}O as CO_2, etc.		
1	1	Standard 6-60-RMS/TA system per P.L. Pubs 1927	109,865.	
2	1	Replace 6-60 analyzer by 12-90	13,500.	
3	1	Substitute type III RMS/SU collector for standard	4,000.	
4	1	Electron multiplier power supply	1,500.	
5	1	Turbopump (170 l/s), instead of standard source ion pump	3,000.	
6	1	Automatic reservoir-pressure-change flow control system	6,175.	
7	1	Substitute "interpolative" inlet system (3 reservoirs, 3 leaks) for standard	2,350.	
		Sub-total		140,390.
		B. 3-60-HD for HH/HD as H_2		
1	1	Standard separate 3-60-HD analyzer per PUBS 1927, Item 2B	20,000.	
2	1	Substitute electromagnet (without supply) for std. permanent magnet	-0-	
3	1	Plenum to permit pumping of 3-60 source by turbopump of A5 above	1,000.	21,000.
		C. Shipping and insurance		1,250.
		Total		162,640

QUOTATIONS FIRM FOR 30 days

TERMS ☐ NET 30 DAYS
☒ See Terms of Payment

SHIPMENT
☐ FOB STATE COLLEGE, PENNA.
☒ FOB Destination (C)
☐

NUCLIDE CORPORATION

E. J. Puchalla

BY: E. J. Puchalla
Marketing Coordinator

FOR FURTHER INFORMATION CONTACT:
Dr. L. F. Herzog

ITEM	QUANTITY	DESCRIPTION	UNIT PRICE	EXTENSION
		II. Augmentations for the thermionic source isotope analysis of K as KCl and Rb, Ca etc. as suitable salts		
		A. Source		
1	1	Sliding bar "SU" vacuum lock source instead of standard, add	15,000.	
2	1	LN_2 trap for V.L. source	1,500.	
3	1	EB ionizer module for 1	4,000.	
4	1	Gas inlet for 3 and modifications to 1 to facilitate "docking" it	1,500.	
5	1	Valve to isolate 12-90 source from pumping plenum	1,750.	
6	1	Valve to isolate 3-60 source from pumping plenum	1,250.	
		Total		25,000.
		B. Collector of 3-60-HD		
1	1	Substitute "dual dual" collector to convert to 3-60-RMS/HD (which can also measure isotope ratios of CO_2, N_2 etc.)	2,500.	
		C. Most strongly recommended accessories and spares		
1	1	Interchangeable second carriage for sliding-bar vacuum lock,with source HV section module	5,500.	
2	4	Metal "hats" for individual filaments	90.	360.
3	1	Glass button triple-filament adapter	250.	
4	2	Boxes (25 ea.) of glass button triple filament mounts with Re filaments	250.	500.
5	1	"Triferential pumping" - 25 l/s ion pump added near collectors (of 12-90)	3,500.	
		Total		12,610

ITEM	QUANTITY	DESCRIPTION	UNIT PRICE	EXTENSION
		D. Other Options		
		1. For preparing filaments		
1	1	Spotwelder, capacitance type, for attaching filaments	3,250.	
2	1	Standard filament preconditioning system	12,500.	
		2. To complete 3-60-RMS/HD electronics		
		a1. MR-18C magnet supply/regulator, current regulated only	5,000.	
		-or-		
		a2. MR-20 magnet supply/regulator with both field and current regulation	9,500.	
		b. MEC-4, master electronics control, with dual preamps (EAH-400) for 3-60-RMS/HD	9,450.	
		Delivery: 4-6 months ARO		
		Terms of Payment: See attachment entitled "Progress Payment Terms		

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